

Methods: The swabs from the different parts of foot: second toe-web and onych were cultured and identified according to standard mycological procedures (bioMérieux; Sanofi Pasteur, France). The sensitivity to antifungal agents was tested using ATB Fungus (bioMérieux) and Fungitest (Sanofi Pasteur) tests.

Results: Fifty swabs from 25 patients with filarial lymphedema, hospitalized in Varanasi-India were cultured. Sixty nine different strains of fungi (19 species) were isolated. Among 69 isolates 56.6% of yeast fungi, 24.6% of moulds and 18.8% of dermatophytes were identified. *Candida* and *Geotrichum* species dominated among yeast fungi. These species were cultured mostly from onych-swabs (71.2%). Among moulds dominated *Aspergillus* sp. The growth of *Absidia* sp. were also observed. The resistance to itraconazole were determined among isolated strains.

Conclusions: The fungal colonization of skin in patients with filarial lymphedema may be an important reason for chronic inflammatory disorders.

Tup20 *Histoplasma capsulatum* endocarditis during AIDS: Case report and review

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Background: Disseminated *Histoplasma capsulatum* (Hc) infection is a frequent complication of advanced HIV-infection among subjects who reside(d) in endemic areas. Most commonly extra-pulmonary sites affected by Hc include the hematopoietic system and skin. We report a case of disseminated Hc infection with a tricuspid valve endocarditis during AIDS.

Case report: A disseminated Hc infection with confirmed pulmonary, cutaneous and oropharyngeal involvement was diagnosed in a 39-year-old Haitian woman with AIDS (CD4 = 2/mm³). A transesophageal echocardiography (TEE) revealed small vegetations of the tricuspid valve with leaflet perforation. Intravenous amphotericin B (total dose = 2 g) and subsequent maintenance therapy with itraconazole (400 mg/d) resulted in a complete clinical recovery. Repeated TEE disclosed a complete regression of tricuspid vegetations within 15 months. Hc infection did not relapse during 24 months of follow-up.

Discussion: According to a literature review, only 42 cases of Hc endocarditis have been reported previously. Most cases (94%) occurred in association with clinically disseminated disease, including oropharyngeal and laryngeal involvement in 42%. Only one of these patients had an underlying immunodeficiency and Hc endocarditis has never been reported before in HIV-infected subjects.

Conclusion: Endocarditis should be considered a potential localization of disseminated Hc infection in AIDS. It appears that prolonged remission of tricuspid endocarditis can be obtained with antifungal therapy alone.

Tup21 Lamisil 1% cream in the treatment of pityriasis versicolor

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Objective: In assessment of therapeutic efficiency and local tolerance of 1% terbinafine cream among patients with superficial dermatomycoses, 29 patients with Pityriasis versicolor were treated.

Methods: Diagnosis was established by clinical and mycological examination. In this study 16 men and 13 women, age 18–60 were evaluated. Disease lasted from 2 months up to 2 years. Terbinafine 1% cream was applied once a day, for two weeks. Therapeutic efficiency was estimated for 30 days, on the 0 and 15 day of therapy and on the 15 day after therapy was discontinued. Assessment of therapeutic efficiency was based on clinical parameters (itching, scaling, redness, vesicles, inflammation, exudation, maceration, fissures) and mycological examination. Depending on severity, those parameters were graded from 0–3. Score of the disease was result of the summation of numerical values. Clinical efficiency of applied therapy was assessed by the decrease of the score, and mycological cure was assessed by positive/negative mycological finding. Mycological evaluation was performed by native microscopic examination.

Results: Results are showing decrease of the clinical score (76.30%) on the 15th day of therapy, and mycological cure in 82.35% patients. That means higher mycological cure rate than clinical cure rate. In the follow-up period

(15 days after therapy was discontinued), clinical cure rate raised to 87.05% and became almost equal with the mycological cure rate. There were no side effects.

Conclusion: Terbinafine 1% cream in the treatment of Pityriasis versicolor once a day for 2 weeks, results in high clinical and mycological cure rate, without side effects.

P:3/2 – Fungal infections - laboratory

Tup23 Resistance of *Candida albicans* in biofilm to antifungal agents

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We studied the tolerance of *C. albicans* (CA) biofilm to amphotericin B (AMP), itraconazole (ITZ), miconazole (MIZ), ketoconazole (KTZ) and fluconazole (FLZ), using a polystyrene tube model. Briefly, 24 h culture of CA on SAB agar was standardized in YNB, supplemented with 50 mmol of dextrose, to give 1 to 5 × 10³ CFU/ml. Two ml portions of the medium were transferred to the tubes and incubated at 37°C. After 24 h, the contents of the tubes were aspirated, the biofilms were washed 3 times with PBS and the planktonic cells were collected, washed and standardized in fresh YNB (group 1). The biofilm tubes were divided into two groups, one was reconstituted with 2 ml of YNB (group 2), and the other was dislodged by sonication in 2 ml ice cooled YNB (group 3). The antifungal agents were added at two fold serial dilutions (0.0325–8 µg/ml) to the tubes. After another 24 h incubation, the biofilms of group 2 were dislodged by sonication and the OD of all tubes were measured at 625 nm and compared to that of drug-free tubes. The IC50 (minimum concentration required to inhibit growth by 50%) was determined for each drug. The biofilm of CA showed resistance to the antifungal agents (IC50 range 4 to > 8 µg/ml) compared to the planktonic cells (0.5 to > 8 µg/ml) and to the sonicated cells (0.25 to > 8 µg/ml). AMP showed highest activity against the three phases – IC50 of 0.5, 0.25, and 4 µg/ml against the planktonic, the sonicated cells, and the biofilm, respectively. ITZ was the most active azole (IC50 of 2, 0.5 and > 16 µg/ml). Our results show that CA in biofilm is resistant to antifungal agents which may account for the lack of response in device related infections. These isolates retain sensitivity to the antifungals when tested in the planktonic phase.

Tup24 Detection by ELISA of *Candida* mannan and anti-mannan antibodies in sera from haematological patients

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Objective: To evaluate the interest of two new ELISA for the detection of *Candida* mannan and anti-mannan antibodies, in the survey of *Candida* infections in patients with haematological malignancy.

Methods: During a one year prospective study, 1035 and 1011 sera from 141 patients hospitalised in haematology unit were analysed respectively with Platelia *Candida* Ab and Platelia *Candida* Ag (Bio-Rad).

Results: Antidody detection assay gave positive results for 3 patients; all sera of these 3 patients remained positive during the survey. These results were confirmed in haemagglutination assay. The detection of mannan was positive several times for 4 patients and only one time for 3 others patients. Invasive candidosis can be suspected for 5 of these 7 patients. The positivity of antigenemia is related to a reduction or a stopping of the Amphotericin B treatment or prophylaxis. Only 3 patients gave positive haemoculture, all due to *Candida glabrata*. Diagnosis of septicemia could be made for 2 of these 3 patients but neither antigen or antibody was detected in their sera.

Conclusion: Due to the efficiency of prophylaxis, the number of confirmed deep infections was too low to allow the confirmation, in a prospective study, of the good sensitivity previously obtained in retrospective studies. We put forward the good specificity of these two assays in this population.

TuP25 *Candida* mannan antigen and antibodies detection in liver transplant patients

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Objectives: To evaluate the interest of two new enzyme immunoassays for detecting circulating antigen from *Candida* and anti-mannan antibodies in liver transplant recipients.

Methods: 67 sera from 15 patients submitted to a liver transplant were analysed by Platelia *Candida* Ag (Bio-Rad). This ELISA, using anti mannan EBCA1 monoclonal antibody, allows the detection of 0.25 ng of mannan per ml of sera. The quantification of anti-mannan antibodies was performed by Platelia *Candida* Ab (Bio-Rad) in 65 sera from the same patients.

Results: For one patient, invasive candidosis was diagnosed after isolation of *Candida albicans* in haemoculture. This patient was positive with the two ELISA. Mannan was detected in the sera of four others patients. For two of them mannanemia occurred only the day after transplant and became rapidly negative. In the third patient, the positivity of Ag went with an infectious syndrome with unknown origin and with several abdominal operations. In the last patient, the positivity of Ag was replaced by the increasing of antibodies against mannan after treatment with Amphotericin B.

Conclusion: The ELISA mannan Ag and Ab assays promises to be sensitive methods to help the establishment of the diagnosis of candidosis. Definite validation in transplant patients should be determined in a larger prospective study.

TuP26 Oral candidiasis in patients under actinotherapy: Molecular identification and typing of *Candida famata*

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Objectives: To establish, compare and qualify methodologies capable of detecting, identifying and typing emerging oral yeast pathogens to head and neck actinotherapy recipients.

Methods: Oral swabs from 71 patients undergoing actinotherapy for treatment of malignant head and neck tumors were examined microscopically following culture onto CHROMagar[®] *Candida* (Paris, France) in order to screen for *Candida* species. All presumptively identified isolates were identified according to their carbohydrate assimilation profile by the API ID 32 system (bioMérieux, Marcy l' Etoile, France). Molecular typing of *C. famata* isolates followed by PCR and restriction fragment length polymorphism analysis (RFLP).

Results: *C. albicans* was responsible for 63.3% whereas *C. glabrata*, *C. tropicalis*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, *C. inconspicua*, *C. famata* and *Clavispora lusitanae* constituted the remaining 36.7% of the isolates. Oral infection of most patients (67.6%) was due to a single *Candida* species, 5.6% due to two *Candida* species and 2.8% due to three *Candida* species. *C. famata* differentiation from *C. guilliermondii*, which give a doubtful carbohydrate assimilation profile, was facilitated by PCR with primers from the LSU rDNA of *Debaryomyces hansenii*. *C. famata* strain differentiation was deduced through distinct *MspI* restriction maps of the ITS1/4 PCR products.

Conclusions: CHROMagar[®] is a rapid screening medium for presumptive identification of non-*C. albicans* species. *C. famata* can be accurately and promptly identified and typed by a combination of rapid methods, ensuring successful control of mixed candidal infections.

TuP27 Mannan antigen and antibodies in haematological patients with invasive candidiasis

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Objectives: *C. tropicalis* is one of the species commonly involved in candidemia. The objective of this study was to establish the usefulness of mannan antigen and antibodies detection in the follow up of haematological patients with proven invasive candidosis due to this species.

Methods: 64 sera from 5 patients with proven invasive candidiasis due to *C. tropicalis* were retrospectively analysed by Platelia *Candida* Ag (Bio-Rad) a monoclonal based ELISA, allowing the detection of 0.25 ng of mannan per ml of sera. The detection of anti-mannan antibodies was performed on the same sera by Platelia *Candida* Ab (Bio-Rad).

Results: *C. tropicalis* was isolated from blood culture of the 5 patients. Cutaneous samples, liver biopsy and CSF were also *C. tropicalis* positive for respectively 4, 1 and 1 patients. High levels, up to 5 ng/ml, of mannan were found in the sera of the 5 patients. The positivation of Platelia *Candida* Ag occurred at the same time or earlier (up to 9 days) than blood culture. Research of anti mannan antibodies by Platelia *Candida* Ab was positive for 4 patients. This assay was well correlated with Co-electrosynerese assay.

Conclusion: The study of the variations of the concentration of mannan and anti-mannan antibodies allows to improve the diagnosis, in particular its precocity, of *C. tropicalis* invasion and to support the management of the patients.

TuP28 PCR for *Pneumocystis carinii* in different immunosuppressed and immunocompetent patient groups

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Objectives: To determine the value of single and nested PCR for *Pneumocystis carinii* pneumonia (PCP) diagnosis in different immunosuppressed and -competent patient groups with acute respiratory diseases using different respiratory specimens (sputa, endotracheal aspirates, lung biopsies and bronchoalveolar lavage [BAL] specimens).

Methods: 574 respiratory samples of 334 patients with acute respiratory diseases (89 HIV-positive patients, 61 transplant recipients, 66 malignancy patients, 34 otherwise immunosuppressed and 84 immunocompetent patients) were prospectively examined by microscopy, single and nested PCR. Resulting data were correlated with clinical evidence of PCP.

Results: For the detection of PCP cases among HIV-patients using BAL specimens, microscopy and single PCR were 100% sensitive and specific, while nested PCR although 100% sensitive reached a specificity of only 97.5%. In the three different immunosuppression groups, both PCRs produced lower positive predictive values (PPV) than microscopy in BAL samples. Among immunocompetent patients, the PPV of both PCRs was 0. Furthermore, specificities and PPVs in all patient groups and for all differing specimens examined were lower for nested PCR than for conventional microscopy.

Conclusions: The diagnostic value especially of nested PCR does not seem to offer additional advantage as compared to conventional microscopy. However, nested PCR identified a significant percentage of clinically silent *P. carinii* colonization in 17 to 20% of immunocompetent and immunosuppressed non-HIV-patients. The observation that *P. carinii* colonization without clinical PCP in HIV-patients was virtually not found may suggest that PCP does usually not result from reactivation of latent infection during immunosuppression.

TuP29 Lipid peroxidation by alveolar macrophages challenged with *Cryptococcus neoformans*, *Candida albicans* or *Aspergillus fumigatus*

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Objectives: To investigate whether the enhanced production of oxygen radicals by alveolar macrophages (AM) stimulated with *Cryptococcus neoformans*, *Candida albicans* or *Aspergillus fumigatus* cause cellular lipid peroxidation (LPO) or/and peroxidation of lung surfactant phospholipids.

Methods: The level of malonaldehyde, an indicator of LPO, was determined by the Biotex LPO-586 colorimetric assay. AM damage was examined by electron microscopy (EM), trypan blue exclusion and counting the AM loss from culture dish to supernatant.

Results: Stimulation of AM by each fungus increased cellular LPO but did not affect AM viability. A slight surfactant LPO by AM alone was shown with significantly increased values after addition of each fungus. Dense lipid droplets, presumably oxidized lipids were ingested in high amounts together with *C. neoformans* and in low amounts with *C. albicans* as shown by EM.

These processes were accompanied by increased numbers of AM in the supernatants.

Conclusions: In the lungs, AM exposed to one of these fungal pathogens might cause peroxidation of surfactant lipids.

TuP30 In vitro activity of antifungal agents to *Candida* spp.

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Objectives: the representatives of a genus *Candida*, isolated from genital tract of the women with various inflammatory.

Methods: isolation and the identification were conducted on generally accepted methods, sensitivity determined by a method MIC.

Results: was inspected 382 women. 82% from a total number *Candida* spp. made *Candida albicans*, 11% - *Candida crusei*, 5% - *Candida tropicalis*, 2% - *Torulopsis* spp.

95% of the strains *Candida albicans* were sensitive to natamycin, 70% - are sensitive to nystatin, 65% - to fluconazole, 58% - to clotrimazole, 97% - to ciclopiroxolamine. 97% *Candida crusei* were stable to fluconazole. 91% of the strains were sensitive to natamycin, 65% - to nystatin, 54% - to clotrimazole, 96% to ciclopiroxolamine. For *Candida tropicalis* and *Torulopsis* spp. is marked the sensitivity to natamycin for 94% of the strains, to nystatin for 66%, to clotrimazole for 67%, to ciclopiroxolamine for 98%.

Conclusions: thus natamycin and ciclopiroxolamine are most active in relation to *Candida* spp.

TuP31 In vitro susceptibility of antifungal agents against *Candida* species by the fungitest

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94 strains of *Candida* spp. (45-*C. albicans*, 13-*C. tropicalis*, 18-*C. krusei*, 14-*C. kefyr*, 2-*T. glabrata*, 1-*C. parapsilosis*, 1-*C. stellatoidea*) isolated from the blood, throat, stool in patients with hematologic diseases were tested for antifungal agents (amphotericin B - AB, fluconazole - Flu, itraconazole - Itr, 5-fluorocytosin - Fc, ketoconazole - Ket,) by the Fungitest (Sanofi Diagnostics Pasteur). It is a micromethod in RPMI liquid medium, using a standardized inoculum, (10^3 CFU mL⁻¹).

Results:

Species (n)	MIC (mcg mL ⁻¹)									
	AB		Flu		Itr		Fc		Ket	
	2	8	8	64	0.5	4	2	32	0.5	4
<i>C. albicans</i> (40),%	98	100	60	78	40	65	88	88	55	65
<i>C. tropicalis</i> (13),%	100	100	85	85	54	77	77	92	69	85
<i>C. krusei</i> (18),%	83	89	0	17	33	72	22	78	50	67
<i>C. kefyr</i> (14),%	100	100	65	100	57	86	57	100	86	93
<i>T. glabrata</i> (2)	2/2	2/2	0/2	0/2	1/2	1/2	2/2	2/2	0/2	2/2
<i>C. parapsilosis</i> (1)	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
<i>C. stellatoidea</i> (1)	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1

5 strains of *C. albicans* were isolated repeatedly. Three of the five *C. albicans* developed resistance to fluconazole, the susceptibility to other drugs was not changed.

Evaluation of the in vitro activity of antifungal agents against *Candida* species is necessary in patients with hematologic diseases. The susceptibility of *Candida* species may change during the antifungal therapy.

TuP32 Analysis of *Candida* isolates from blood cultures

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Objectives: The aim of this study was to analyze the incidence of *Candida* species in blood cultures collected from patients in our hospital.

Methods: The study involved the patients hospitalized at the Public Hospital No 1 in Gdansk from 1 January 1997 to 31 December 1999. The samples of blood were examined using BacT/Alert System (Organon

Teknika). The identification of *Candida* species was done with the VITEK system (bioMérieux) using YBC card.

Results: In total, 4516 blood cultures were positive, including 132 fungal isolates from 61 patients (3% of the total number of samples). The isolation of different species of *Candida* from blood, was the following: *C. parapsilosis* - 53, *C. albicans* - 41, *C. glabrata* - 12, *C. tropicalis* - 4, *C. krusei* - 4, *Trichosporon beigelii* - 3, and four others *Candida* species - 14. 61 patients (55 adult/6 children) with candidemia were evaluated. The following species were isolated: *C. parapsilosis* in 21 patients, *C. albicans* in 21, *C. glabrata* in 7, *C. krusei* in 3, *C. tropicalis* in 2, *Trichosporon beigelii* in 1 and others four species *Candida* in 6. Majority of isolates were from: Adult's Hematology Unit patients - 72, Pediatrics - 30, surgical patients - 14, Intensive Care Unit - 13 and 3 from patients others unit. Incidence *C. parapsilosis* isolation from blood increased from 7 in 1997 to 19 in 1998 and 27 in 1999 and *C. albicans* from 8 in 1997 to 17 in 1998 and 16 in 1999.

Conclusion: *C. parapsilosis* and *C. albicans* were the most frequent species isolated and the highest incidence of fungemia was in Adult's Hematology Unit.

TuP33 Karyotyping of *Candida albicans* and *Candida glabrata* isolates from recurrent vaginal infections by pulsed-field gel electrophoresis

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Objective: To apply pulsed-field gel electrophoresis (PFGE) based the chromosomal DNA fingerprinting in the clinical microbiology laboratory for karyotyping of *Candida albicans* and *Candida glabrata* isolates from recurrent vaginal infections.

Methods: 16 women with recurrent vulvovaginal candidiasis (RVVC) due to *Candida albicans* and *Candida (Torulopsis) glabrata* were followed for a period of 4 to 12 months, and 36 *Candida* isolates were typed by PFGE.

Results: 11 of the women were infected with *C. albicans* and 5 with *C. glabrata*. Three electrophoretic karyotypes of *C. albicans* and three of *C. glabrata* were identified throughout the follow-up. All but one patient were infected with the same karyotype of *C. albicans* or *C. glabrata* during this period. Two different karyotypes of *C. glabrata* were identified in one patient over 12 months. The results confirmed the diversity of the karyotypes of *C. albicans* and *C. glabrata* causing vulvovaginitis, and demonstrated the persistence of colonization with the same strain over different periods of time despite therapy (15/16 women).

Conclusions: The PFGE method can be used for a more precise differentiation of different *Candida* species and for the karyotyping of yeasts, including *C. albicans* and *C. glabrata*, for subspecies determination.

TuP34 Deep dermatophytosis associated with tacrolimus in a renal transplant recipient

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Deep dermatophytoses (DD) are a rare manifestation of superficial mycoses. In some cases they have been associated with immunocompromised hosts. We report a case of DD infection caused by *Trichophyton rubrum* (yellow form). A 63 yo caucasian male received a cadaveric renal transplant for end stage renal failure due to adult polycystic kidney disease. He was CMV D-R + and initial immunosuppression was with cyclosporin A, azathioprine and prednisolone. Two weeks post transplantation (Tx) he developed an episode of acute steroid resistant rejection requiring OKT3. His immunosuppressive regimen was altered and tacrolimus substituted for cyclosporin A. Post Tx he had no serious infective complications but he was treated for a superficial mycosis of fingernails with tioconazole. *Trichophyton rubrum* was isolated and the infection clinically improved. Two years post Tx the patient presented with 6 nodules extending over both lower limbs which had rapidly increased in size over 5 weeks. Biopsy revealed necrobiotic granuloma and fungal hyphae on histological PAS staining and *Trichophyton rubrum* (yellow form) on culture. He was treated with liposomal amphotericin B (~total dose of 5 g) and then itraconazole solution with therapeutic drug monitoring, for 2 months. Repeat excision biopsy after 3 weeks of therapy revealed residual fungal hyphae. His clinical condition improved

and no new lesions developed. Literature review illustrated this is a rare complication of Tx immunosuppression and there is no clear consensus on duration of treatment. We suggest the need for aggressive management of

superficial mycoses both pre and post Tx as well as prolonged treatment of established infections.

Topic 4 – Bacterial infections

O:4 – Bacterial infections

Mo07 Bacterial infections induce a stimulus-related transcription of the calcitonin precursor gene *CALC-1*

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Objective: *CALC-1* gene transcription gives rise to a family of circulating peptides including thyroid C-cell calcitonin (CT) and several calcitonin precursors (CTpr). In systemic infections, there is massive elaboration of CTpr but no corresponding increase in CT. Furthermore, CTpr per se is toxic to animals and immunoneutralization improves their survival. The origin of CTpr remains to be clarified.

Methods: A hamster model of lethal *E. coli* peritonitis was used for peptide (RIA) and CT mRNA (RT-PCR and in situ hybridization) determinations.

Results: In septic animals, high molecular weight CT (i.e., CTpr) was produced in a variety of organs (e.g., liver, lung, kidney) as well as being detected in the serum. By RT-PCR, CTmRNA was expressed in a variety of septic tissues but absent in most healthy control tissues. In situ hybridization revealed the presence of CT mRNA in many tissues and cell types.

Conclusions: In experimental sepsis, CT gene expression is ubiquitously distributed. CTpr constitute a diffuse network of related peptides that, in the presence of systemic infection, are preferentially mobilized and discharged in a stimulus-specific manner. Importantly, since our prior studies showed that the immunoneutralization of CTpr enhances survival, uncovering the molecular mechanisms regulating *CALC-1* transcription may reveal novel treatment approaches to sepsis.

Mo08 Regulation of toxin production in *Clostridium difficile* is connected with its energy metabolism

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Background: Toxin positive strains of *C. difficile* may differ up to 100 000-fold in toxin production, but the mode of regulation of its two (toxin A and B) genes is not known. Toxin production is up regulated by biotin limitation and in glucose free rich media, and down regulated by addition of glucose or a mix of 9 growth promoting amino acids.

Material and Methods: Production of toxins, metabolic end products (short chain fatty acids, alcohols, acetone) and cell protein patterns of various *C. difficile* strains during different growth conditions were studied by immunoassay, GLC and 2-D PAGE, respectively. PCR amplified toxin gene promoter DNA bound to magnetic Dynabeads was used for affinity isolation of promoter binding proteins. Proteins were identified by N-terminal amino acid sequencing and data base searches.

Results: Among the 9 amino acids cysteine was the most potent suppressor of *C. difficile* toxin production both *in vitro* and *ex vivo* (in diluted human feces). Up regulation of toxin production was associated with switched on synthesis of several metabolic enzymes plus an electron carrier protein and with a changed pattern of metabolic end products. The enzyme 3-OH-butyryl-CoenzymeA-dehydrogenase was not only up regulated but also bound to the toxin A and B gene promoters (but not to unrelated DNA) during high toxin production, linking a change of bacterial metabolism with toxin expression in *C. difficile*.

Conclusion: We hypothesize that certain metabolic stress leads to altered metabolism, ATP and end product levels, and switching on the toxin A and B genes in *C. difficile*, whereas overnutrition of the organism has the opposite effect. Administration of e.g. cysteine to the large bowel may become a new approach to prophylaxis and therapy of *C. difficile* diarrhea.

Mo09 Eap (extracellular adherence protein), an adherence enhancement protein from *Staphylococcus aureus*

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Objectives: To investigate the role that Eap plays in the process of *S. aureus*'s binding to host cells.

Methods: Purification of Eap was done from *S. aureus* supernatants using affinity chromatography followed by ion exchange chromatography. Adherence of Eap to fibroblasts, epithelial and endothelial cells were measured by ¹²⁵I-labelled Eap. Adherence of *S. aureus* to fibroblasts and epithelial cells was done for one hour onto confluent layers of cells. Binding was determined microscopically.

Result: The binding of *S. aureus* to host tissues is multifactorial. Plasma binding proteins produced by the bacteria play a crucial role for the attachment of *S. aureus* to the host. Eap (extracellular adherence protein) a plasma binding protein contribute to this process. Eap form oligomers, rebind and agglutinate the bacteria. This protein can bind to seven plasma proteins among which we found fibronectin, fibrinogen and prothrombin. Eap binds to eukaryotic cells with approx. 7×10^6 Eap molecules/cell. The adherence enhancement resulting from Eap is dose dependent and increase the binding of the bacteria 5 times.

Conclusion: The enhancement of adherence seen in this study is due to the ability of Eap to bind both to host plasma proteins and to the surface of *S. aureus*. It thereby forms a bridge between the eukaryotic cell and the bacterium.

Mo010 Surveillance of invasive pneumococcal disease in the US Arctic and sub Arctic: Alaska 1986–1998

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Objectives: Surveillance of invasive pneumococcal disease was established in Alaska in 1986 to: 1) evaluate rates of disease in both Native and non Native segments of the population, 2) determine the serotype distribution, in preparation for vaccine programs, and 3) monitor and control antimicrobial resistance.

Methods: Invasive pneumococcal isolates collected between 1986 and 1998 from Alaska Natives and non Natives from 23 hospital laboratories were serotyped, and tested for antibiotic susceptibility by agar dilution. More than 40% of the 1620 sterile site isolates were from Alaska Natives which constitute <20% of the total population.

Results: Invasive disease rates based on culture were 62 and 16 cases/100,000 respectively for Native and non Natives. Serotyping confirmed that 92% were contained in the 23 valent vaccine. Among Native and non Native children < 2 years of age rates were 450 and 129/100,000 respectively. Serotypes in the 7 valent conjugate vaccine were recovered from 73% and 83% of isolates from Native and non Native children < 2 years respectively. Overall reduced susceptibility to penicillin (MIC > 0.1–< 2 ug/ml) was found in 10.5% of isolates, and 3.2% were fully resistant (MIC > 2 ug/ml). In 1986 only 2.5% of isolates had reduced penicillin susceptibility and none were resistant. However in 1998, 6.8% had reduced susceptibility, and an additional 6.2% were fully resistant.

Conclusions: High rates of invasive pneumococcal disease are found in the Alaska Native population, and the emergence of antibiotic resistance in this population now complicates treatment. Efforts to reduce the disease burden and control antibiotic resistance include continued surveillance, use of vaccines, and education of communities and health care providers on appropriate antibiotic use.